experimental data, and predicts the cellular volume and pressure for several models of cell cortical mechanics. Furthermore, we show that when cells are subjected to an externally applied load, such as in an AFM indentation experiment, active regulation of volume and pressure leads to complex cellular response. We found the cell stiffness highly depends on the loading rate, which indicates the transport of water and ions might contribute to the observed viscoelasticity of cells.

2454-Pos Board B473

The Mechanics of Phagocytic Cups Measured in Real-Time using Magnetic Particles

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Phagocytosis has been extensively studied in search for biochemical pathways that drive the reorganization of the cell membrane and the subjacent cytoskeleton during the internalization of a phagocytic target. This active deformation makes phagocytosis an inherently mechanical process. However, a characterization of the process in terms of the underlying measures such as energies and forces remains elusive.

Here, we present a novel approach to measure the stiffness of a phagocytic cup and relate it to the energy stored in it. We allow magnetic particles coated with immunoglobulins to bind to human macrophages and trigger Fc-receptor mediated phagocytosis. As the membrane progressively wraps around the particle, we quantify the concomitant changes in stiffness by applying a time-varying magnetic field and measuring the translational and rotational excursions of the particle [Irmscher et al., Biophys. J., 2012]. The shear stresses are applied orthogonal to the direction of particle uptake and do not disturb the physiological rate of particle internalization.

The measurements reveal that the stiffness initially rises slowly as the particle is increasingly engulfed by the membrane. The stiffness eventually peaks and proceeds to drop to a lower level. We explain this characteristic evolution of stiffness by invoking a mechanical model that treats the phagocytic cup as a pre-stressed membrane in connection with an elastically deformable actin cortex. Under this minimal description, the observed peak in rotational stiffness marks the point when half of the particle is engulfed. The rate of stiffness increase before the peak is conserved across cells and indicates that the membrane advances at a characteristic speed.

Our approach paves the way for studies on the mechanical effects of recently discovered uptake inhibitors (such as CD47) that are relevant in cancer immunology.

2455-Pos Board B474

'Marker of Self', CD47, Modulates Mechanical Forces Imposed by Macrophages during Phagocytosis

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Macrophages are innate immune cells, which engulf and digest pathogens and opsonized cells. This uptake process, known as phagocytosis, is actin-driven and becomes more efficient in the presence of phospho-activated non-muscle myosin IIA (NMMIIA). CD47 is a transmembrane membrane protein which acts as a marker of self by inhibiting phagocytosis by macrophages. An IgG-opsonized target cell or particle lacking CD47 binds macrophage FcR which then activates the assembly of paxillin, F-actin, and nonmuscle myosin IIA at the phagocytic synapse. In contrast, parallel interactions with CD47 signal through SIRP to inhibit myosin assembly and contractile contributions to efficient phagocytosis. We have studied the force and kinetics of phagocytosis by human macrophages. To this aim, we studied phagocytosis of opsonized red blood cells. The force imparted during phagocytosis was calculated based on blood cells' shape deformation and elastic properties. Our analysis shows that the range of the resultant force imposed by Macrophages is up to 100pN and in 10 minutes after initial contact. Further we show that CD47 modulates the force generated by macrophages on phagocytic targets.

Muscle: Fiber and Molecular Mechanics & Structure II

2456-Pos Board B475

Molecular Mechanical differences between Skeletal Muscle α-Actin and Smooth Muscle γ-Actin in the Presence of Smooth Muscle Tropomyosin

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Molecular mechanical differences between actin isoforms remain elusive potentially because their sequence differences are limited to the actin regulatory protein binding sites[1]. We executed *in vitro* motility measurements of smooth muscle myosin with skeletal muscle α-actin (α-Act) and smooth muscle γ-actin (γ-Act) alone and in complexes with smooth muscle tropomyosin (Trop/α-Act and Trop/γ-Act, respectively) and extracted mean sliding velocity (v), motile fraction (f_{mot}), sliding velocity coefficient of variation (CoV), mean non-motile time (t_{stop}), and mean motile time (t_{run}) as functions of actin length (L). We found a discrete separation of actin sliding into a motile and a non-motile population; the motile fraction increases with L. Characteristic lengths (L_c) above which $f_{mot} \geq 75\%$ are: α-Act 2.86[2.23,4.01] n=10, γ-Act 2.52 [1.95,3.29] n=11, Trop/α-Act 1.57[1.25,1.93] n=10, and Trop/γ-Act 3.36 [2.52,5.04] n=11 (μm, [95% confidence interval], n flow through chambers). Thus, L_c is statistically significantly reduced for Trop/α-Act vs. all other conditions ($p<0.05$), whereas Trop/γ-Act displays a trend towards an L_c increase compared with α-Act and γ-Act. Further, Trop/α-Act v is elevated ≈1.2-fold over all other conditions, f_{mot} is elevated ≈1.12-fold over Trop/γ-Act, CoV is reduced to ≈0.82-fold of the Trop/γ-Act value. We constructed a mathematical model of the actomyosin kinetics which captures the L-dependent bimodality, v, f_{mot}, and CoV and predicts the t_{stop} and t_{go} L-dependence. In steady state analysis as well as stochastic simulations only a myosin detachment rate increase for Trop/α-Act can explain the above results. Our findings suggest a regulatory protein-dependent, actin isoform-specific regulation as the mechanism underlying the correlation of actin isoform ratio and smooth muscle phenotype[2].

[1] Harris, Warshaw, Circul Res, 72(1):219-24, 1993 [2] Szymanski et al., Am J Physiol Cell Physiol, 275:C684-92, 1998; Funding: CIHR, NIH RO1-HL 103405-02, NSERC

2457-Pos Board B476

Influence of Actin Mutation on the Energy Landscape of Actin-Tropomyosin Filaments

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The energy landscape describing the movement of tropomyosin on the actin filament was determined computationally for a set of tropomyosin positions on the surface of wild-type and mutant actin (K326N, E334K, D25N). All the mutations studied, have been detected in individuals with various myopathies and analysis of changes in the energy-landscapes may provide insights into disease mechanisms. The landscape for wild-type actin-tropomyosin is characterized by a broad energy basin with a discrete minimum whose shape is dominated by electrostatic interactions between actin and tropomyosin. Previous studies indicate that the energy minimum localizes tropomyosin near to the filament "blocked-state" in these troponin-free filaments (Li et al., 2011). The K326N mutation in actin leads to a ~40% lower electrostatic interaction energy and a shift in the position of the energy minimum toward the "open-state" tropomyosin position. This is consistent with the increased Ca^{2+}-sensitivity found for corresponding mutant muscles (Jain et al. 2012). In case of the E334K mutation, the low-energy basin seems to be more diffuse than it is in the wild-type but the overall strength of the actin-tropomyosin interaction is greater. Additionally, a small shift of the equilibrium structure toward the open-state is observed, while the electrostatic-interactions energy minimum does not show a major shift. These observations suggest that tropomyosin may oscillate to a greater extent about its equilibrium position on E334K actin. In contrast to the influence of K326N and E334K, D25N mutation does not affect the energy landscape diagram in any obvious way. Here, the landscape displays features that are almost identical to that of wild-type, consistent with the hypothesis the mutant impacts myosin- rather than tropomyosin-binding to actin (Wong et al. 2002; Sparrow et al. 2003).

2458-Pos Board B477

Actomyosin Interactions and Different Structural States of Actin Filaments

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The persistence length (L_P) of a polymer is proportional to its flexural rigidity and quantifies the decay length of its tangent angle (for a polymer freely